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A new *Hysterostomella* species from the Cerrado in Brasília National Park

C.A. Inácio*, R.C. Pereira-Carvalho, E.S.C. Souza, H.B. Sales & J.C. Dianese.

Departamento de Fitopatologia, Universidade de Brasilia, Distrito Federal, 70.910-900, Brazil
* Correspondence to: *inacio@unb.br*

Abstract — A new *Hysterostomella* species found on leaves of *Connarus suberosus* is illustrated and described as *Hysterostomella connari*.

Key words — *Ascomycota*, biodiversity, *Parmulariaceae*, taxonomy, tropical fungi

Introduction

Parmulariaceae, including 34 genera of foliicolous biotrophic fungi, occurs primarily in the Neotropics and Paleotropics (Inácio & Cannon 2008, Kirk et al. 2008). The type genus of the family, *Parmularia* Lév. and its type species *P. styracis* Lév., was described on *Styrax ferrugineus* (Léveillé1846), a plant species endemic to the Brazilian Cerrado. For decades this family was set aside with occasional contributions (Müller & von Arx 1973, von Arx & Müller 1975, Barr 1987, Sivanesan 1970, Sivanesan & Hsieh 1989, Sivanesan et al. 1998). Recently the type species of all accepted genera were re-examined, consolidating our understanding of the family on the basis of morphological features (Inácio 2003, Inácio & Cannon 2003, 2008). Now a new species belonging to the genus *Hysterostomella*, previously observed by Sales (2003) and Silva et al. (2006) from the Brazilian Cerrado, is formally described and illustrated.

The revision by Inácio (2003) of *Hysterostomella* Speg., type species *H. guaranitica* Speg., included a re-description of several species. The genus is characterized by subcuticular internal stromata when host cells are incorporated by the infective mycelium. Sometimes these structures are subepidermal, connected to superficial stromatic ascomata by peg-like or tubular hyphal filaments. The discoid multilocular stromatic ascomata contain spherical or elongate locules dispersed in various orientations (Inácio 2003, Inácio & Cannon 2008). *Hysterostomella* is close to *Cycloschizon* Henn., which differs by

forming internal stromata that become erumpent via a column with a wart-like expanded top containing locules distributed in complete or incomplete rings. *Dothidasteroma* Höhn., also close to *Hysterostomella*, differs mainly by forming flat internal stromata, never subepidermal, that connect to the superficial ascomata by either bundles of dark colored hyphae, or single hyphae.

Materials & methods

Leaf samples of *Connarus suberosus* containing dark brown to black crustose fungal structures were collected from different areas of Cerrado in Parque National, Brasília. These were pressed and dried for 3–4 days at ± 50°C, and deposited in the Mycological Collection of the Herbarium UB. The leaves were observed under a stereomicroscope to describe the lesions and location and characteristics of the fungal colonies. For the morphological studies, each specimen was mounted on slides using lacto-glycerol/cotton blue or glycerol KOH/basic phloxine, to observe the internal structures. Pieces of the dried material containing stromata were re-hydrated for 4–12 h using an aqueous solution containing 10% ethanol and 0.1% Tween-20. Then 7–15 µm thick sections were produced using a freezing microtome (MICROM GMBH, HM 500 OM, Micron Laborgeräte, Walldorf, Germany). A compound light microscope (Zeiss Ultraphot III, Carl Zeiss, Oberkochen, Germany) was used for the photographic documentation and to obtain morphometrical data. Pieces of leaves with one or more lesions showing representative samples of fruiting bodies were examined with SEM after treatment in a 0.1 M sodium cacodylate buffer (pH 7.4) containing 2% glutaraldehyde for at least 24 h. The samples were dehydrated in an increasing acetone concentration series (15%, 30%, 50%, 75%, 100%; 15 min per concentration). Leaf pieces were then dried at the critical point before being covered by a thin layer of gold in a sputter coater for 2 min. Finally, the samples were observed in a scanning electron microscope (Jeol, model JSM 840-A E).

Taxonomy

Hysterostomella connari Inácio, Pereira-Carvalho, E.S.C. Souza, H.B. Sales & Dianese **sp. nov** Figs 1–21

MycoBank MB 561556

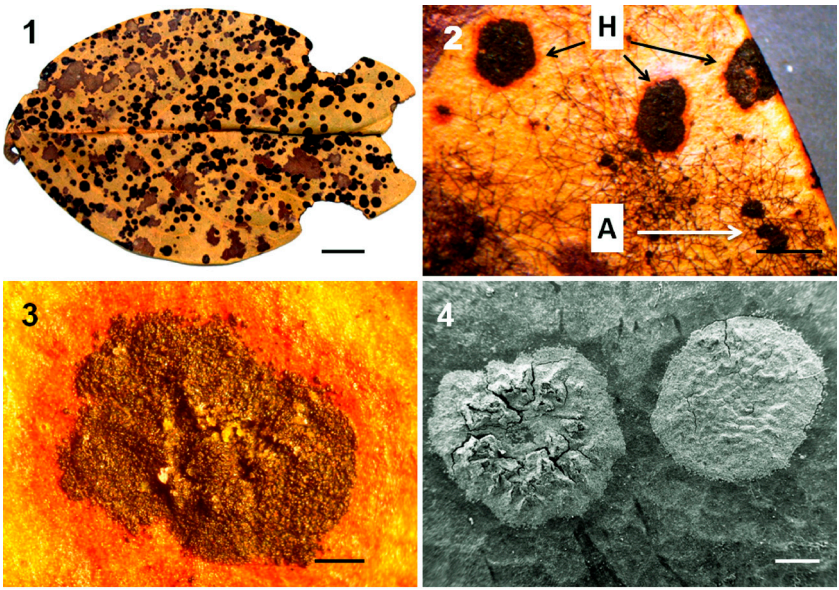
Species haec ab Hysterostomella tetracerae *differt conidiis non fasciatis et grandioribus.*

Type: BRAZIL. Distrito Federal, Brasília, Parque Nacional, near Parada do Cristal, on leaves of *Connarus suberosus* Planch. (*Connaraceae*), 18 Jan. 2010, leg. *Carlos Antonio Inácio* (**holotype**, UB Mycol. Coll. 21492).

Etymology: referring to the host genus.

Lesions 3–12 mm diam, black, circular encircled by a reddish linear band, adaxially more evident. Colonies sparse, containing black circular or irregular superficial stromata that are discoid, mostly epiphyllous, crustose, sheltering conidiomata, spermogonia and/or ascomata.

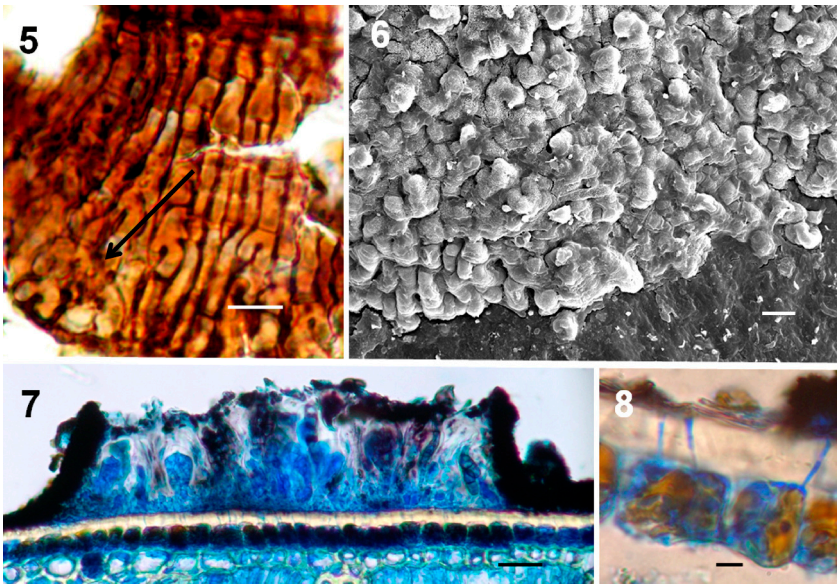
Internal pseudostromata as mycelium with colourless to reddish-brown angular cells invading and incorporating one or more host cell layers, to 30 µm



Figs 1–4. *Hysterostomella connari* on leaves of *Connarus suberosus*. 1. Adaxial colony shown as a group of stromatic ascomata circular to irregular in shape. 2. Crustose stromatic ascomata (H) together with a colony of an *Asterina* species (A). 3. A stromatic ascoma bordered by a reddish band (bar). 4. An older opened ascoma (left) and another still closed (right) seen in SEM. Scale bars: 1 = 10 mm; 2 = 2 mm; 3, 4 = 0.5 mm.

deep. Superficial stromata 0.1–3.5 mm diam., 50–113 × 150–680 µm in vertical section, subepidermal, erumpent, brown to dark brown, shiny, with laciniate edges; upper surface composed of textura prismatico-radiata, the hyphae with brown prismatic cells that proliferate dichotomously towards the margins to form a circular or irregular 1–2 cell-layered multilocular shield that houses the sexual and asexual fruiting bodies; rather prominent, opening by irregular slits; upper wall 10–30 µm thick, brown to dark brown, with surface consisting of 5–7 µm diam brown cells; lower wall 30 µm thick, brown to dark brown, covering the host epidermal cells, dense, bearing a basal cushion of mingled colourless, light brown to brown hyphae, bearing the asci or lining the internal surface of the conidiomata and spermogonia. Mycelium internal. Hyphae colourless to pale brown, 1–2 µm diam, septate, branched, penetrating and occupying the epidermal and first layers of mesophyll, sometimes with coiled intracellular structures; to 100 µm deep.

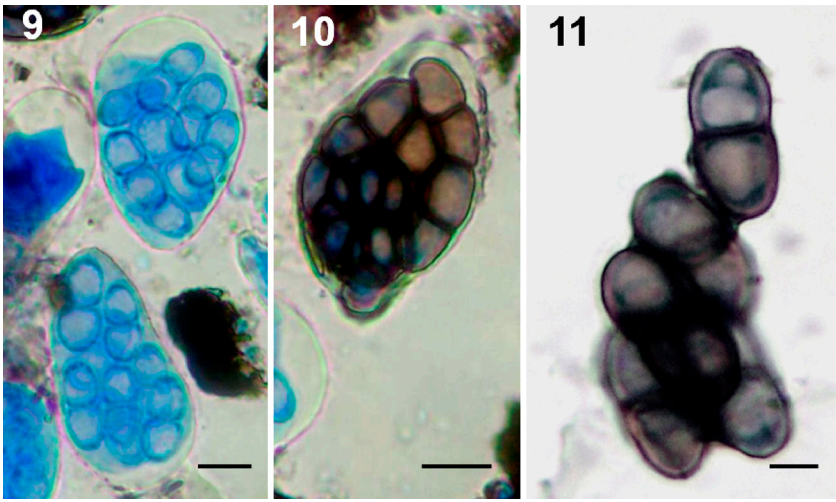
Conidiomata included in the superficial stromata, gregarious, intermixed with the ascomata and spermogonia, globose to subglobose, opening by diametral or irregular fissures, uni- or multilocular, 40–160 µm high in vertical



Figs 5–8. *Hysterostomella connari* on leaves of *Connarus suberosus*. 5. Superficial textura of a stromatic ascoma, with arrows showing the dichotomous proliferation of component hyphae. 6. Surface of an ascoma seen under SEM. 7. Vertical section through an ascoma showing parallel asci and branched sterile filaments, and transcuticular hyphae connecting the flat portion of the pseudostroma with the superficial ascoma. 8. Detail of the interconnection between the components of the internal pseudostroma with the superficial ascoma. Scale bars: 5, 6, 8 = 10 µm; 7 = 50 µm.

section, with a brown to dark brown upper wall; upper wall dense, 10–25 (–30) µm thick, composed of textura prismatico-angularis; cells 3–7 µm diam.; conidiomatal locules 25–150 × 120–610 µm, single or sometimes multiple separated by a stromatic wall, with conidiogenous cells and sterile filaments attached to the inner face of the upper locular wall; conidiogenous cells 8–20(–25) × 3–7 µm, ampulliform, cylindrical to lageniform, colourless to pale brown, smooth, holoblastic; conidia 9–16 × 6–10 µm, initially colourless, becoming pale brown to brown, guttulate, obovoid to widely clavate, aseptate, smooth, with a truncate base. Spermogonia structurally and dimensionally similar to the conidiomata; spermogenous cells 2–7 × 2–3 µm, phialidic, hyaline, cylindrical; spermatia 3–5 × 1–2 µm, aseptate, hyaline, bacilliform.

Ascomata 50–95 × 155–580 µm, intra-stromatic, occurring separately from, or mixed with conidiomata and/or spermogonia occupying the same stromatic crust, containing parallel asci and sterile filaments on a basal cushion of colourless to light brown hyphae covered by a thin gelatinous layer; young asci variable in shape, cylindrical, clavate to broadly clavate, with a short



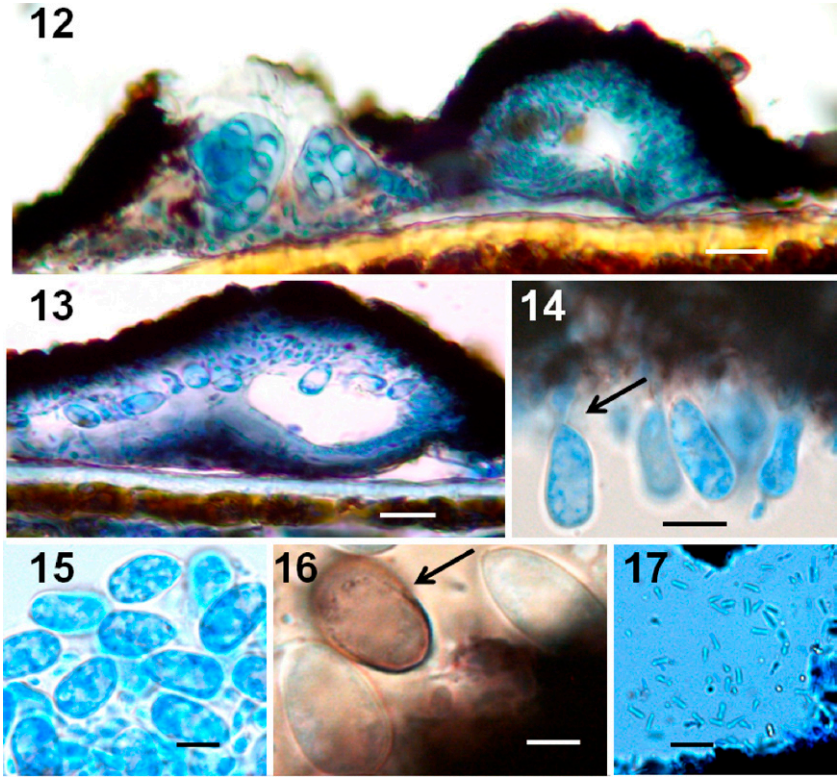
Figs 9–11. *Hysterostomella connari* on leaves of *Connarus suberosus*. 9. Immature asci. 10. A mature bitunicate ascus with dark brown ascospores. 11. Ascospores. Scale bars: 9–11 = 10 µm.

pedicel, thick-walled particularly towards the apex where, before spores are visible, a sub-apical chamber is present; FULL-SIZED ASCI showing 60–70 × 18–30 µm, 6- to 8-spored with ascospores arranged in one to three rows, broadly-clavate, thick-walled particularly in the upper part, collapsing after spore release showing a large apical crack in a typically fissitunicate fashion; ASCOSPORES 17–21 × 7–11 µm, becoming light to dark brown, 1-septate, guttulate, covered by a mucilaginous sheath, ellipsoidal, round at both ends, narrower at the septum, tapering towards the lower tip; INTERASCAL FILAMENTS septate, smooth, colourless to light brown, thin-walled, 2–4 µm diam, filiform, round or slightly attenuate at the tip, branched above the middle portion, immersed in a gelatinous matrix.

ADDITIONAL SPECIMENS EXAMINED: **BRAZIL. DISTRITO FEDERAL, BRASÍLIA, Parque Nacional**: 2 km North from Parada do Cristal—on leaves of *Connarus suberosus*, 24 Jan. 2007, leg. Rafael Guimarães, collection number 6 (UB Mycol. Coll. 20.420); Parada do Cristal´s pathway—on leaves of *Connarus suberosus*, 06 Feb. 2006, Siomara Vasconcelos da Silva (UB Mycol. Coll. 20.115); Unknown sites within Parque Nacional—08 Jan. 1996, Zuleide Martins Chaves 302 (UB Myc. Coll. 10.656) & 321 (UB Myc. Coll. 10.675); 15 Jan.1996, Zuleide Martins Chaves 350 (UB Myc. Coll. 10.738); 24 Jan. 1996, Mariza Sanchez 1476 (UB Myc. Coll. 10.911); 20 Jan. 2010, Gustavo Puga Lemes (UB Myc. Coll. 21529) and Carlos Antonio Inácio (UB Mycol. Coll. 21516)

Discussion

Inácio (2003) and Inácio & Cannon (2008) have partially revised *Hysterostomella*. All species are assumed to show clear host specificity, and no



Figs 12–17. *Hysterostomella connari* on leaves of *Connarus suberosus*. 12. An ascoma (left) beside a spermogonium (right). 13. A conidioma with a typically inverted hymenium. 14. Holoblastically formation of a conidium (arrow). 15. A group of immature hyaline conidia. 16. Mature dark brown aseptate conidium (arrow). 17. Spermatia. Scale bars: 12, 13 = 50 µm; 14, 15, 17 = 10 µm; 16 = 5 µm.

Hysterostomella species has previously been recorded associated with members of the *Connaraceae*. Among the species with re-examined type materials, only *H. tetracerae* (Höhnel 1909) and *H. gymnosporiae* (Hansford 1947) have a conidial anamorph similar to that found in *H. connari*. However both differ because their conidia have a clear hyaline median band. In the remaining species there are no records of anamorphs. Spermogonia as shown in *H. connari* were previously reported only in *H. tetracerae,* which, however, differs additionally by its larger ascomata (88–188 µm diam.), darker and easily discernible lower ascomatal wall, and narrower asci. *Hysterostomella gymnosporiae* forms more prominent ascomata (170–250 µm high) and more restricted internal pseudostromata than *H. connari*.

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